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Transplantation Experiment of Lichen Thalli of *Parmotrema tinctorum* (Ascomycotina, Parmeliaceae)

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Transplantation of square fragments of thalli was successfully performed for *Parmotrema tinctorum*. The fragments were covered with pieces of nylon mesh stapled to the substratum and were cultured on the trunks of *Cryptomeria japonica* under natural condition. Numerous granules, cylindrical protuberances and lobules were formed on the periphery of the fragments 15 months after transplantation. The granules and cylindrical protuberances seemed to have been originated from the upper cortex and medulla, including algal layer, of the transplanted fragments and resemble isidia formed on the upper surface. They were fused each other to form obovate or palmate dorsiventral protuberances and lobules in later stages. Lecanoric acid and atranorin were produced in the newly developed lobules.

Key words: Nylon mesh, *Parmotrema tinctorum*, regeneration of lobules, transplantation.

Parmotrema tinctorum (Nyl.) Hale is a common foliose lichen growing on various trees and rocks in temperate and subtropical zones, including eastern and south-western Japan. It is extremely sensitive to air pollution (Sugiyama 1973, Sugiyama et al. 1976) especially to sulfur dioxide, a by-product of the burning of fossil fuel (Hawksworth and Rose 1970, Sugiyama et al. 1976). Therefore, it has been used as a bioindicator of air pollution in Japan, while it has been threatened or extinct around and in certain urban or industrial area.

Since lichens have a dual nature, asexual propagules such as soredia, isidia, schizidias, fragments of thalli, etc. which include fungal and algal partners together, can grow directly into new thalli. Shuster et al. (1985) reported early growth and development of soredia of *Hypogymnia physodes* and *Physcia tenella*. Besides, Honegger (1995, 1996) reported that the foliose lichen *Xanthoria parietina* (L.) Th.Fr. was capable of regenerating new lobes along wound margins or cut edges of the thallus by culturing on artificial ceramic substrata. On the other hand, the transplantation experiments with corticolous lichens were reported by Brodo (1961), Hallingback (1990) and Armstrong (1994). Bando and Sugino (1995) tried to cultivate *Parmotrema tinctorum* in growth cabinets. However, they reported only the influences of environmental elements such as humidity, temperature, pH etc. on the growth of the lichens. In these studies, regeneration and morphogenesis of transplanted thalli have not been

observed.

Through the present study, transplantation of thalli of *Parmotrema tinctorum* was successfully performed. Numerous lobules were formed on the periphery of transplanted fragments. The regeneration and morphological changes of granules into lobules observed in the transplanted fragments will be reported in the present paper.

Material and Methods

Fragments (7 × 7 mm) were cut off with a knife from marginal part of thalli of *Parmotrema tinctorum* (Nyl.) Hale collected at Kiyosumi, Chiba Prefectrure. These fragments were transplanted on the trunks of *Cryptomeria japonica* D. Don growing near by the collecting site. These transplanted fragments were covered with pieces of nylon mesh about 2 × 2 cm in size, which were stapled to the bark at the corners (Fig. 1A). Average temperature (mid summer, mid winter and annual) and annual rainfall at the experimental site were obtained from monthly report of meteorological data compiled by the Experimental Station for Forestry of the Tokyo University and is shown in Table 1.

For light microscopic observation, sections were made by using a freezing microtome and mounted in GAW solution. Naturally dried specimens were cemented on specimen-holders, sputter coated with about 100Å gold, and examined at 15 kV in a JEOL JSM- 5410LV scanning electron microscope. New lobules formed on the periphery of the fragments were chemically tested by the standardized thin-layer chromatographic method (Culberson 1972).

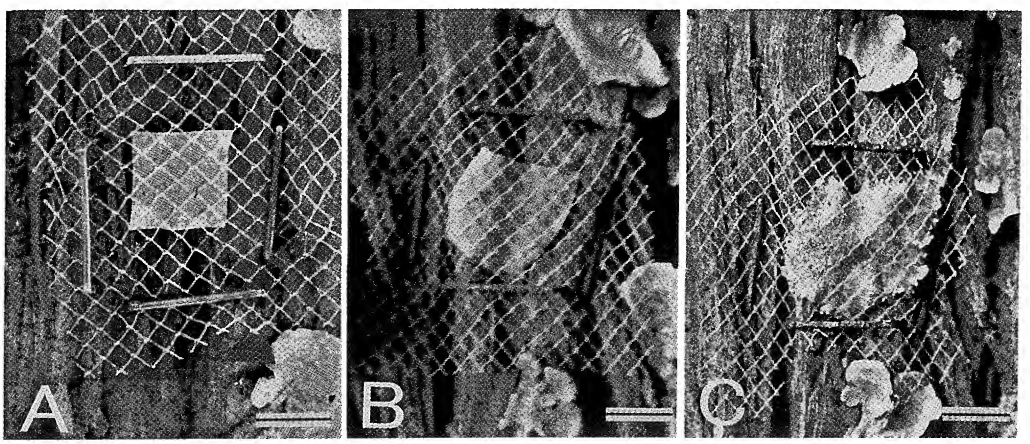

Fig. 1. Transplantation of fragments of thalli of *Parmotrema tinctorum*. A: A fragment transplanted on trunk of *Cryptomeria japonica* on 5 May 2001. B: A fragment 4 months after transplantation on trunk of *Cryptomeria japonica*. No lobule was formed. C: A fragment 15months after transplantation on trunk of *Cryptomeria japonica*. Numerous juvenile, granules, protuberances and lobules were formed. Scale = 5 mm.

Table 1. Average temperature and annual rainfall at the experimental site

Average temperature			Annual rainfall
mid summer	mid winter	annual	2000–2400 mm
25 ℃	4 ℃	14.2 ℃	

Results and Discussion

Although the transplanted fragments of thalli increased the area to about 69 mm^2, no remarkable morphological change was observed 4 months after transplantation (Fig. 1B). Fifteen months after transplantation, however, both the increase of an area 83 mm^2 and remarkable morphological changes were observed. As shown in Figs. 1C, and 2A, numerous granules, protuberances and

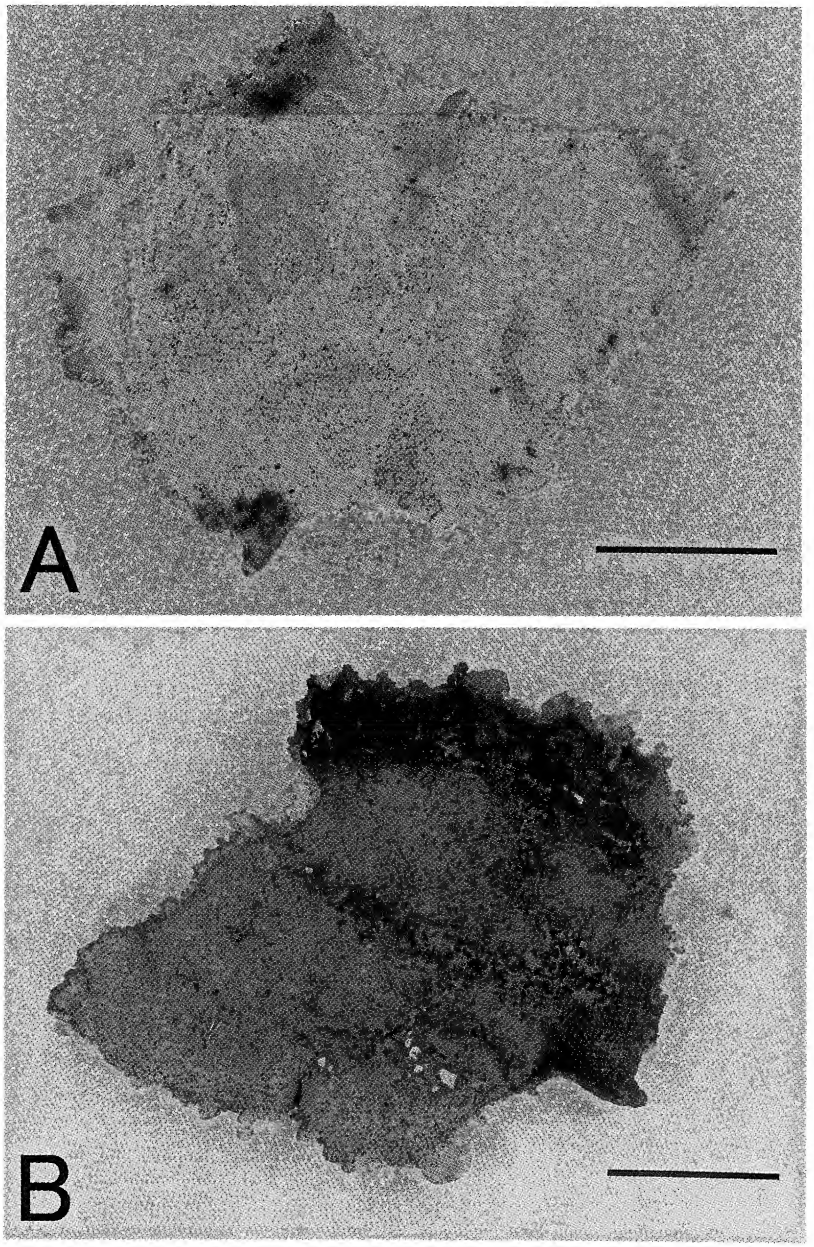

Fig. 2. A fragment 15 months after transplantation. A: upper view B : lower view. Scale = 3 mm.

lobules were found on the periphery of the fragments. In addition, numerous juvenile isidia were found on the upper surface and some rhizines were observed on the blackened lower surface (Figs. 2B, 3B). It should be noted here that these fragments had taken from the younger marginal parts of the thalli with a pale brown lower surface and with

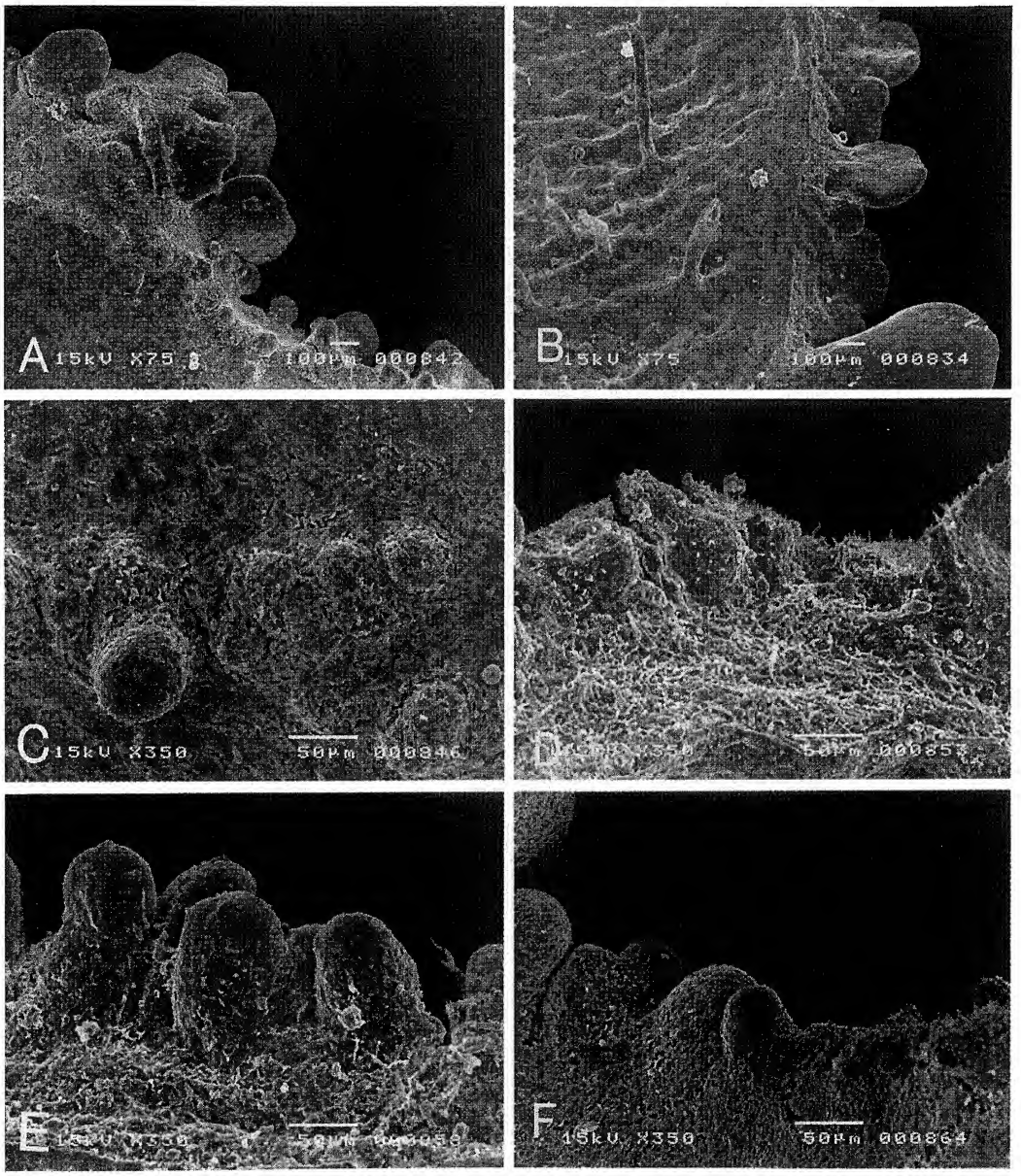

Fig. 3. Scanning electron micrography of fragments 15 months after transplantation. A: lobules formed on the periphery (upper view). B: Newly formed lobules (right) and rhizines (left, lower view). C: Newly formed juvenile isidia on the upper suface of the fragment. D: Globular outgrowths. E: Cylindorical protuberances (lower view). The medulla of the fragment is exposed. F: Cylindrical protuberances (upper view).

neither isidium on the upper surface nor rhizine on the lower surface prior to the transplantation.

As mentioned above, various appendages such as granules, protuberances and lobules are observed on the periphery of the fragments 15 months after transplantation (Figs. 3B, 3D, 3E). Among them, granules can be considered to represent an initial stage of formation of these appendages. The granules resemble juvenile isidia formed on the upper surface (Fig. 3C), being surrounded with cortex. In this stage, continuous cortex could be seen on the upper surface even between the surface of granules and the upper surface of the fragments (Fig. 3F), whereas there were distinct gaps between the surface of fragments and the surface of granules (Fig. 3D) on the lower surface. While the upper cortex of the fragments is connected with that of granules, in other words, the lower cortex of the fragments is not connected with that of granules and the medulla of the fragments was exposed (Figs. 3D, 4A) in this stage. Thus, the cortex of the granules can be considered to have been derived by the growth or extension of upper cortex of the fragments. The granules grew up further to form cylindrical protuberances, in which the cortex is still not connected with the lower cortex of the fragments (Fig. 3E). Therefore, granules and cylindrical protuberances can be considered to have been formed by combined growth of the upper cortex and the medulla, including gonidial layer, of the fragments.

On the periphery of the fragments cultured for 15 months, dorsiventral lobules are also observed (Figs. 2A, 3A, 3B) as mentioned above. These lobules seem to have been formed by the fusion of cylindrical protuberances often becoming dorsiventral in later stages. In part of the fragments, where numerous lobules are formed, lower cortex of lobules was bound with that of transplanted fragments, leaving rather distinct scars along the periphery of the fragments or between newly formed lobules and the fragments

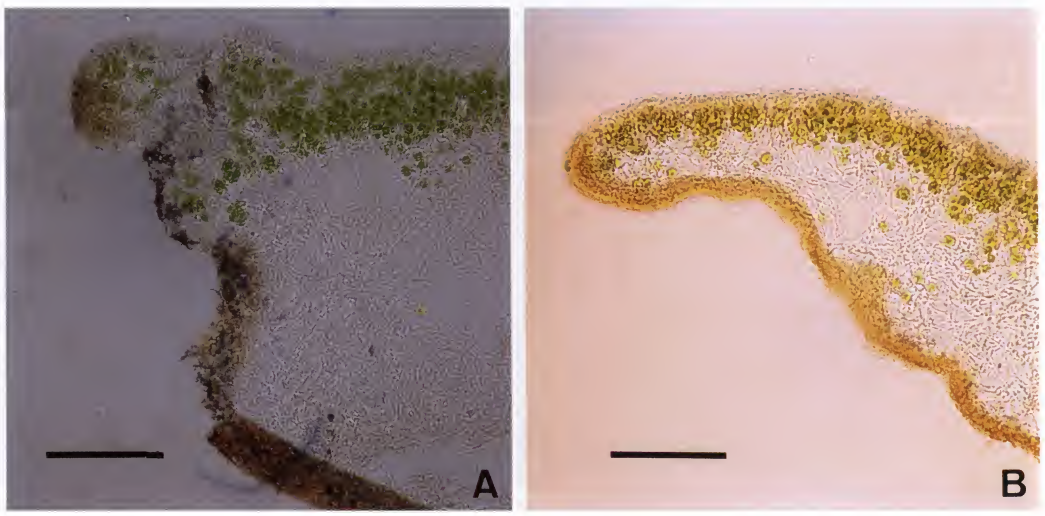

Fig. 4. Cross-section of granule and lobule formed on the periphery of fragment. A: Granules formed on the periphery of fragment. Cortex is connected with upper cortex of fragment but the medulla of fragment is exposed. Scale = 100 μm. B: Lobules formed on the periphery of fragment. Cortex is connected with lower cortex of fragment. Scale = 100 μm.

(Fig. 3B). In dorsiventral lobules, stratified composition similar to original thalli of *P. tinctorum*, though the medulla is rather thin (Fig. 4B). These developmental stages were similar to thallus ontogeny observed in the foliose macrolichen *Xanthoria parietina* (Honegger 1993).

Fifteen months after trarnsplantation, newly formed lobules were cut off from the fragments and the secondary products of them were tested by TLC. The results of TLC tests clearly showed the presence of atranorin and lecanoric acid, which both are produced in nature.

As shown above, transplantation of thallus fragments of *P. tinctorum* was successfully performed using nylon mesh. Appendages such as granules, protuberances or lobules in various stages of regeneration were observed on the periphery of the transplanted fragments. At present, we do not know what the triggers are for regeneration of granules or protuberances on the periphery and for the morphogenesis of granules into lobules. The transplant technique introduced here may be useful for further studies on regeneration and morphogenesis of lichen thalli. It may be also useful in studies of lichen ecology and physiology, including studies on influences of air pollution using transplant method of lichen.

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近　芳明[a]，嶺田美智子[b]，柏谷博之[c]：ウメノキゴケの移植実験

ウメノキゴケの地衣体周辺部分から切り出した，7 mm 四方の地衣体小片をスギ樹皮にナイロンメッシュで固定し，15か月培養したところ，地衣体小片の周辺部分から多くの新しい小型の地衣体（lobule）の再分化が観察された．Lobule 形成の過程は以下のようである．形成の初期段階においては，藻類を含む皮層もしくは髄層部分から小突起状のものが形成される．これらは，上皮層から形成される isidia と形態がよく似ている．さらに，この小突起は円筒形状に伸長し，やがて，これらはお互いに融合し手のひら状の lobule が形成される．ウメノキゴケには二次代謝産物として，レカノール酸とアトラノリンが含まれるが，新しく形成された lobule にも同じ 2 成分が検出された．

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